REMARKS/ARGUMENTS

Claims 7 to 9 are deleted. Claim 4 was previously deleted. Upon entry of the amendment the claims will be 1-3, 5, 6 and 10.

A declaration under Rule 132 by the declarant Taro Aoki, one of the Applicants is enclosed. It is discussed below.

Claim 10 is amended to depend from Claim 1.

The specification is amended to correct obvious typographic errors at pages 4 and 10.

Claim 10 is also amended to correct the spelling of "eicosapentaenoate".

Re: Claim Objections

In view of the deletion of Claims 7-9, the objection to them is now moot.

The criticized spelling in Claim 10 is corrected.

Re: Claim Rejection – 35 U.S.C. § 103

Reconsideration and withdrawal of the rejection of retained Claims 1-3, 5 and 10 under 35 U.S.C. § 103(a) as being unpatentable over Nakamura et al. (Int. J. Clin. Lab. Res. reference cited by applicants; see IDS filed May 26 , 2004) in view of Applicants' acknowledgement at page 4, line 13 – page 5, line 1 of the specification and Fujikawa et al. (U.S. Patent No. 5,856,336) for the reasons of record are requested.

Applicants retain their position that the teachings of Nakamura et al. are limited to the two HMG-CoA reductase inhibitors (statins) that were involved in their observations. The Official Action refers to the employment by Nakamura et al. of the plural term "inhibitors" in various locations. This is appropriate when the authors are discussing prior work, but in discussing the work reported in the publication, there were only two such inhibitors involved, pravastatin and simvastatin. Generalization beyond that would seem to be speculative.

Nakamura et al. simply disclose the inclusion of an EPAE treatment with apparently, a continuation of the two different statin treatments. There is no comparison with a control which would be continued statin treatment alone. There is therefore insufficient basis in the Nakamura reference for the conclusion that all statins (HMG-CoA inhibitors) would be usefully combined with EPA for treatment purposes.

And again, Nakamura et al. do not disclose the clinical data for their patents before they entered the statin treatment regimen. To reach a proper conclusion of the effectiveness of the combination, it would also be necessary to have conducted a trial with EPA alone.

Applicants on the other hand have disclosed, as was previously stated, that their results are synergistic which is factually supported, witness Fig. 1, to which Applicants refer at page 10. As detailed in the second paragraph at page 10, a statistical procedure was followed to establish that there was less than five chances in 100 (P values less than 0.05) for the combination of therapeutics to yield the result reported which is therefore significantly different from application of pitavastatin alone, confirming the synergistic effect.

Such a result should be satisfactory to support unobviousness for the reason that the prior art contains nothing definite upon which to have a conclusion concerning what the prior art would have expected for the combined therapies.

This is a key point. The theoretical rationale for the drug effects does not yield quantitative predictions. To require that Applicants carry out a program to establish such results would obviously, be an impractical burden. Applicants have stated that they have shown a synergistic effect for the combined drug group according to accepted statistical procedures. That should be enough, it appears to Applicants.

As to the requirement that Applicants explain how the date support a conclusion of non-obviousness, it is respectfully submitted, as noted above, that this has been done, since the observed "p" value was less than the 5% considered to be significant (page 10, lines 9 and

10). This means that the hypothesis that there is no difference in the result actually observed from that expected (the standard null hypothesis) has less than 5% chance of being true for the observed results. Therefore, the alternative hypothesis that a statistically significant difference exists is confirmed. Please see page 142, near bottom of column 2, of the Remington citation previously submitted.

Re The Rule 132 Declaration

The herewith submitted Aoki declarant under Rule 132 supplies the numerical values for the disclosed test results shown graphically in Fig. 1 of the specification; please see page 10, last paragraph.

Concerning the matter of demonstrating a synergistic result based upon the above data, it is observed that under the law of mass action and the expression for an equilibrium constant of a chemical reaction, one would consider ratios of dosages and of effects (rather than additive effects, note for example the treatment at pages 82-84, 89, 92 and 93 of Goldstein et al. Principles of Drug Action, 2nd Ed, 1974, copy attached, particularly the discussion of "the fraction of maximal response", pg. 92.

Applicants urge that a synergic effect is recognized when the TG relative value (% to control) for the combined use of pitavastatin and EPA is lower than the product of TG relative values for single use of each of pitavastatin and EPA. Since the data shown in the submitted declaration satisfy this criterion, a synergic effect is confirmed.

That result follows from the calculation summarized in the following table, the data being that from the declaration.

Table 1 (corresponding to Fig. 1)

	Triglyceride in blood (mg/dL)	Relative value (%)
Control	68 ± 6	100.0
Pitavastatin calcium	58 ± 6	85.3
EPA-E	49 ± 5	72.1
Pitavastatin calcium + EPA-E	41 ± 2	60.3 (<61.5)

(P<0.01)

This table shows that the above expected numeral value 61.5 is the product of the observed TG value to control values of [0.853 (pitavastatin) x 0.721 (EPA-E)] x 100(%) but that the data on the last line for the combined-use of Pitavastatin and EPE-E, which amounts to a relative value 41/68 of 60.3%, is low compared to the expected value of 61.5. Accordingly, for the reason mentioned above, the synergic effect for the combination has been demonstrated.

Please noted that Pitavastatin relative value is obtained from 58/68 = 0.853 and that for EPA-E from 49/68 = 0.721.

In terms of receptor theory one can say that the first named drug reduces the number of available receptors from 100% to 85.3% and the second named drug reduces the remaining receptors to 72.1% of the then available amount, leading to the expected reduction to 85.3% x 72.1% = 61.5%, the order of the reduction being immaterial. The observed reduction is 60.3%, which is significantly lower, establishing synergism. It is assumed that response is proportional to receptor occupancy, Goldstein et al., p. 84.

Favorable reconsideration is solicited.

Respectfully submitted,

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DOCKET NO: 249160US0X

IN THE UNITED STATES PATENT & TRADEMARK OFFICE

IN RE APPLICATION OF	:
TARO AOKI, ET AL.	: EXAMINER: HENLEY, R.
SERIAL NO: 10/780,640	:
FILED: FEBRUARY 19, 2004	: GROUP ART UNIT: 1614
FOR: HYPERLIPEMIA THERAPEUTIC AGENT	:

DECLARATION UNDER 37 C.F.R. § 1.132

COMMISSIONER FOR PATENTS
ALEXANDRIA, VIRGINIA 22313

SIR:

Now come(s) who deposes and states:

1. That Taro Aoki is an applicant of the above-entitled application.

2. That Taro Aoki has been employed by Kowa Company, Ltd. for 13 years as a researcher in the field of pharmacology.

3. That _____ is an applicant of the above-entitled application.

4. That _____ has been employed by _____ for ___ years as a _____ in the field of _____.

5. That _____ is an applicant of the above-entitled application.

6. That _____ has been employed by _____ for ___ years as a _____ in the field of _____.

7. That I (we) have read the Office Action of April 21, 2005 in the above-entitled application and have read and am (are) familiar with each of the references cited in the Office Action by the Examiner.

8. That the experiments described in the Examples on pages 8-10 of the above-entitled application were carried out by the applicants of the above-identified application or under their supervision.

9. In further explanation of the Test Method disclosed in the paragraph bridging pages 9 and 10 of the specification, the following is stated: Blood samples were collected, the total amount of cholesterol (TC) and triglycerides (TG) in blood were assayed. Although the samples differed from each other in TC and TG values, the samples were divided into four groups so that the samples belonging to the same group have similar TC and TG values on average. This grouping procedure was carried out by use of the randomized allocation with the use of the dedicated software (EXSAS version 5).

10. That the numerical values of the statistical analysis of the results of the Test Method described in the paragraph bridging pages 9 and 10 of the above-entitled application and shown graphically in Fig. 1 of the application are as follows:

TABLE 1 (CORRESPONDING TO FIG. 1)

	Triglyceride in blood (mg/dL)	p-value
Control	68±6	--
Pitavastatin calcium	58±6	0.5407
EPA-E	49±5	0.1470
Pitavastatin calcium + EPA-E	41±2**	0.0015

The notation ± indicates the end points at a 99.85% confidence level.

11. The above results confirm the statements concerning the significance the test results set forth in the last paragraph on page 10 of the above application.

12. The undersigned petitioner declares further that all statements made herein of his own knowledge are true and that all statements made on information and belief are believed to be

true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of this application or any patent issuing therefrom.

13. Further deponent(s) saith not.

Jaro Aoki — June 28, 2005
Signature — Date

Signature — Date

Signature — Date

Principles of Drug Action:

The Basis of Pharmacology

SECOND EDITION

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Stanford, California

A Wiley Biomedical-Health Publication

JOHN WILEY & SONS
New York London Sydney Toronto

concentration of insulin for half-maximal effect on glucose uptake is very nearly the same, 6.1×10^{-11} M.

Cells were next disrupted, and membrane fragments were separated from nuclei and from soluble cell components. Insulin binding was found exclusively in the membrane fragments, and all the insulin bound by the intact cell could be recovered in the membranes. Association and dissociation rates were again determined with these preparations, yielding $k_1 = 8.5 \times 10^6$ and $k_2 = 4.2 \times 10^{-4}$; thus a dissociation constant 5×10^{-11} was computed, exactly the same as before. Equilibrium dialysis gave virtually the same result.[175] It seems reasonably certain, therefore, that despite the inability to measure any biochemical response to insulin in broken cell preparations, the specific binding serves as a valid way to label the receptor for further fractionation and isolation.

Finally, material was purified from detergent extracts of liver cell membranes, which could be absorbed to affinity chromatography columns of insulin–agarose. Upon elution, a protein of molecular weight 300,000 was obtained, which bound 1 mole of insulin mole^{-1} of protein, and was virtually pure. Detergents were required throughout the procedure for solubilization of the receptor protein.[183]

The promising results obtained here, as well as those with the AcCh receptor, betoken a significant change in the likelihood of obtaining membrane-bound receptors in pure form. Only 5 years ago the first edition of this book carried the following statement: "Some think that receptors of this type may be impossible, in principle, to be isolated. If separated from the integrated system of which they are a part, they would lose all function, it is argued, and could not even be identified as receptors any longer. Only future research will tell if such pessimism is warranted." Evidently it is not.

CONSEQUENCES OF DRUG-RECEPTOR INTERACTIONS: ANALYSIS OF THE GRADED DOSE-RESPONSE RELATIONSHIP

Application of the Law of Mass Action: The Occupancy Assumption

Biologic responses to drugs are, as a rule, *graded*. They can be measured on a continuous scale, and there is a systematic relationship between the dose (or effective concentration) of a drug and the magnitude (or intensity) of the response it elicits. Application of the law of mass action to the dose-response relationship was largely the contribution of A. J. Clark (1885–1941).[176,177] An observed biologic effect was assumed to be a reflection of the combination of drug molecules with receptors, much as the rate of appearance of products in an enzyme reaction reflects the degree of combination of substrate molecules with enzyme-active centers. The magnitude of a response was postulated to be directly proportional to the occupancy of receptors by drug molecules, with a maximal response corresponding to occupancy of all the receptors. Then equations could be derived from simple mass law principles, describing the dependence of effect upon dose.

Let a drug, X, combine with a receptor site, R, to yield a complex, RX, producing a biologic response of magnitude Δ proportional to the amount (or concentration)

of RX, so that

$$R + X \underset{k_2}{\overset{k_1}{\rightleftharpoons}} RX$$

$$\Delta = k_3(RX)$$

Then at equilibrium,

$$\frac{(R)(X)}{(RX)} = \frac{k_2}{k_1} = K_X$$

where K_X is the dissociation constant of the complex. Then if (R_T) is the total receptor concentration, and since $(R_T) = (R) + (RX)$,

$$\frac{(R_T - RX)(X)}{(RX)} = K_X$$

and, rearranged,

$$\frac{(RX)}{(R_T)} = \frac{(X)}{K_X + (X)}$$

Now let Δ_{max} be the maximal response of which the system is capable, obtained when all receptors are occupied; so

$$\Delta_{max} = k_3(R_T)$$

Then,

$$\frac{\Delta}{\Delta_{max}} = \frac{(RX)}{(R_T)}$$

and therefore

$$\Delta = \frac{\Delta_{max}(X)}{K_X + (X)} \tag{1}$$

This is the familiar hyperbolic function in which $\Delta = 0$ when $(X) = 0$, and Δ approaches Δ_{max} when (X) becomes very large. When half-maximal response is obtained,

$$\frac{\Delta}{\Delta_{max}} = \frac{(X)}{K_X + (X)} = \frac{1}{2}$$

so the concentration of (X) required for half-maximal response is equal to K_X.

The derivation of equation (1) is identical to that of the classical Michaelis–Menten equation, which gives the velocity v of an enzyme reaction as a function of the substrate concentration (S), the enzyme-substrate dissociation constant K_m, and the maximum velocity V_{max}:

$$v = \frac{V_{max}(S)}{K_m + (S)}$$

Here the proportionality $v = k_3(ES)$ refers to the breakdown of a steady-state intermediate, whereas the analogous proportionality $\Delta = k_3(RX)$ is assumed without reference to any particular underlying mechanism. The equations developed here are quite generally applicable; they subsume enzyme-substrate and enzyme-inhibitor interactions as special cases.

Three critical assumptions, which underlie the derivation or usual application of equation (1), may be formulated explicitly, as follows:

1. *Response is proportional to receptor occupancy.* This will be referred to as the "occupancy assumption." In the case of enzyme-substrate and enzyme-inhibitor interactions, since the observed response is determined directly by the concentration of enzyme-bound substrate or inhibitor, the assumption is soundly based. With respect to drug effects in general, however, we are as yet unable to accept or reject the assumption on experimental or theoretical grounds. In a later section we shall discuss alternatives to the "occupancy assumption."

2. *One drug molecule combines with one receptor site.* This is the simplest reaction mechanism, from which equation (1) (and also the Michaelis–Menten treatment) is derived. We shall consider later some molecular combining ratios other than unity.

3. *A negligible fraction of total drug is combined,* so that in equation (1) the term (X), which refers to uncombined drug, may be replaced by (X_T), the total drug concentration. A system in which this assumption is true is said to operate in zone A.[178] In experiments where binding is actually measured, as in equilibrium dialysis, uncombined drug is determined and equation (1) (equivalent to the equations on p. 161) can be used rigorously. However, when a biologic response is the criterion of effect, only *total* drug added to the system is known. It is then usual to assume zone A behavior, as we shall do in the following section (and as is customary in mathematical treatments of enzyme reactions based on the Michaelis–Menten approach). Later, however, we shall examine the consequences when a system does not operate in zone A.

If equation (1) is inverted, the equation of a straight line is obtained:

$$\frac{1}{\Delta} = \frac{K_X}{\Delta_{max}} \cdot \frac{1}{(X)} + \frac{1}{\Delta_{max}} \tag{2}$$

with slope K_X/Δ_{max} and intercept $1/\Delta_{max}$ when reciprocal response magnitudes $1/\Delta$ are plotted against reciprocal doses $1/(X)$, actually $1/(X_T)$, as explained above in connection with the *zone A* assumption.

This procedure is known as a double-reciprocal (or Lineweaver–Burk) plot (Figure 1-66).[179] Increasing drug concentrations are to the left, so the y axis is at $1/(X) = 0$, corresponding to infinite drug concentration, where all receptors are occupied. The y intercept gives $1/\Delta_{max}$. Since the slope, given by equation (2), is K_X/Δ_{max}, K_X could be obtained by estimating the slope; but an easier way is simply to extend the line downward and to the left, as shown. The x intercept will then be $-1/K_X$.

The double-reciprocal plot has been applied widely to the analysis of antagonisms and particularly to enzyme inhibitions, but it has limitations.[180] An antagonist Z is said to be *noncompetitive* if it inactivates the receptor so that the effective complex with agonist X cannot be formed, regardless of the concentration of X. Z might combine with R at the same site where X ordinarily combines, but so firmly that it cannot be displaced. Alternatively, Z could combine at a different site, in such a manner as to prevent a conformation change in R that is essential to its proper combination with X, or that is requisite to producing the characteristic biologic response. Yet again, Z might itself induce a conformation change in R that abolishes the reactivity of the site where X should interact. In noncompetitive antagonism the effects upon receptors

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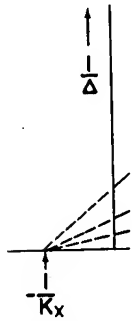

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The Log Dose-Response Curve

In pharmacology it is conventional to show the relationship between dose and response more directly than on a double-reciprocal plot. In Figure 1-70, two methods of graphical presentation are compared. The dose of a glucocorticoid drug was varied, as in the SAR studies described earlier, and the amount of glycogen deposited in the liver was measured. In both cases the dependent variable (amount of glycogen) is plotted on an arithmetic scale of ordinates. On the left the values of the independent variable (dose) are also plotted on an arithmetic scale on the x axis, but on the right these same data are plotted on a logarithmic scale. The curves, which are fairly typical, indicate that from a practical standpoint the logarithmic dosage scale is preferable. Line segments rather than hyperbolic curves are obtained, which are much easier to deal with in statistical analysis. Moreover, drugs that produce the same effect by the same mechanism but differ in potency yield parallel line segments, and this is very convenient. For example, in Figure 1-70b the constant horizontal separation of

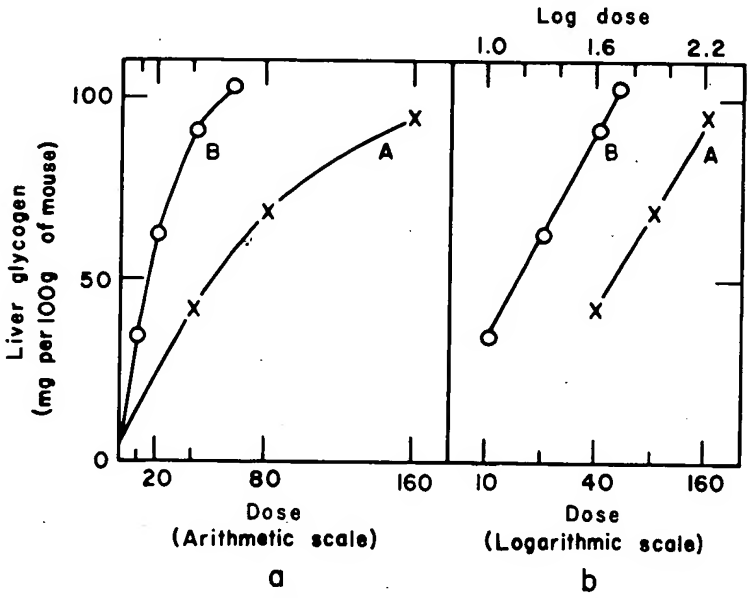

FIGURE 1-70. Linear and semilogarithmic dose-response curves. *Effects of two steroids, 11-dehydrocorticosterone (A) and cortisone (B), on liver glycogen in mice. (From Gaddum, Figure 85[184] adapted from Venning, Figure 2.[185])*

the lines is a measure of the potency ratio for the two drugs, since the difference (log dose A − log dose B) is the same as log (dose A/dose B), where the doses are those required to produce an equal response. Another practical advantage of the logarithmic dosage scale is that a wide range of doses can be presented readily in a single graph. Thus, quite apart from any theoretical considerations, there are sufficient reasons for plotting dose-response relationships on semilogarithmic coordinates, and it has become customary to do so.

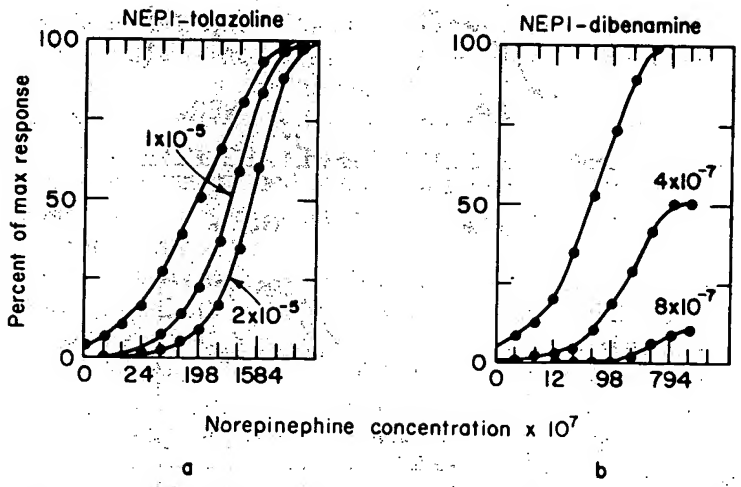

FIGURE 1-73. LDR curves in analysis of antagonisms. *Isolated cat spleen, as in Figure 1-72, stimulated by NEPI at various molar concentrations, as indicated. In (a) and (b) the curve farthest to left is the control, the others were obtained in the presence of two antagonist concentrations. (From Bickerton, Figure 3.*[187])

system (Figure 1-73b) by the effects of two concentrations of dibenamine, an alkylating agent, upon the contractile response to norepinephrine.

Now we shall examine to what extent actual LDR curves can be described by equations based on the law of mass action. Let f be the fraction of maximal response obtained at a given dose, as follows:

$$f = \frac{\Delta}{\Delta_{max}} = \frac{(RX)}{(R_T)}$$

Substituting from equation (1), and rearranging yields

$$f = \frac{(X)}{K_X + (X)}$$

$$(X) = K_X\left(\frac{f}{1-f}\right)$$

By substituting $(X_T) - (RX)$ for (X) and $f(R_T)$ for (RX), we obtain

$$(X_T) = K_X\left(\frac{f}{1-f}\right) + f(R_T) \qquad (3)$$

Total drug = free drug + bound drug

Equation (3) is broadly applicable to dose-response relationships in which the "occupancy assumption" applies, and in which the molar combining ratio of drug to receptor is unity (our first and second assumptions); it does not presuppose any particular fraction of total drug bound to receptors (our third assumption). If in equation (3) the term $f(R_T)$, representing bound drug, is very much smaller than $K_X[f/(1-f)]$, representing free drug, it may be ignored. For any moderate values of f this simplification can be made if (R_T) is small compared with K_X (e.g., R_T/K_X less than 1/10). We will then have the *zone A* approximation, in which practically all

drug molecules are free even at receptor saturation, so that

$$(X_T) \cong (X) = K_X\left(\frac{f}{1-f}\right) \tag{3A}$$

At the other extreme, if R_T is very much larger than K_X, then the expression $K_X[f/(1-f)]$, representing free drug, becomes negligible for any moderate values of f. We will then have the *zone C* approximation, in which practically no drug molecules are free, as follows:

$$(X_T) \cong f(R_T) \tag{3C}$$

Here, because the drug-receptor dissociation constant is so small, or the receptor concentration is so large, the binding is essentially stoichiometric ("pseudo-irreversible") even though the interaction is technically a reversible one. A truly irreversible drug-receptor combination would give the same equation and corresponding LDR curve.

Behavior intermediate between these two extremes, which has to be described by the full form of equation (3), is known as *zone B* behavior. The validity of simplifying equation (3), in the cases of *zone A* and *zone C*, it should be noted, depends entirely upon the ratio R_T/K_X, i.e., upon the concentration of receptors expressed in units of the drug-receptor dissociation constant.

For the same reason equation (3) can be written more usefully in the following way:

$$\frac{(X_T)}{K_X} = \frac{f}{1-f} + f\left\{\frac{(R_T)}{K_X}\right\}$$

The three forms of this equation are plotted as LDR curves in Figure 1-74. Here the x axis shows log dose, each dose being expressed in units of the drug-receptor dissociation constant. Thus f is plotted against $\log (X_T/K_X)$.

The LDR curve for *zone A* in this graph will be recognized as the familiar symmetrical sigmoid, which inflects at $f = 0.5$. Indeed, the zone A LDR curve is identical

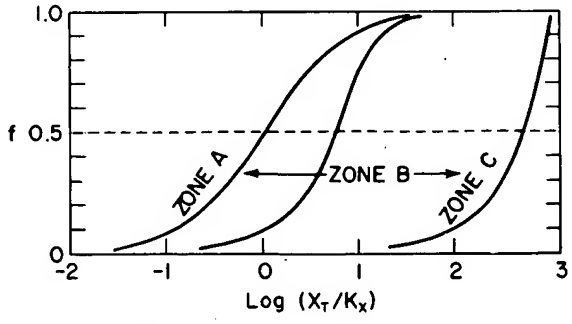

FIGURE 1-74. Theoretical LDR curves for the 3 zones of behavior. *Ordinal values f are fractions of maximal response or fractional occupancy of receptors. Abscissal values are logarithms of drug concentration (dose) normalized by expressing in units of the drug-receptor dissociation constant. In zone A practically all the drug is free. In zone C practically all the drug is combined with receptor sites. In zone B neither free nor combined drug can be neglected; only a single representative curve is shown. (Adapted from Straus and Goldstein, Figure 2.*[178] *By permission of Cambridge University Press.)*